Articles

Synthesis, Monoamine Transporter Binding Properties, and Behavioral Pharmacology of a Series of 3*β*-(Substituted phenyl)-2*β*-(3′-substituted isoxazol-5-yl)tropanes

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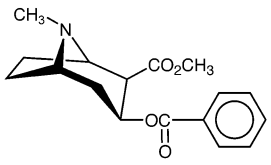
Several 3*β*-(substituted phenyl)-2*β*-(3-substituted isoxazol-5-yl)tropanes (**3a**−**t**) were evaluated for their ability to inhibit radioligand binding at the DAT, 5-HTT, and NET as well as in gross observation and locomotor activity in mice and in rats trained to discriminate cocaine. All compounds showed high affinity for the DAT. The IC_{50} values ranged from 0.5 to 26 nM. With the exception of **3e** and **3f**, which have no substituent on the 2*β*-(1,2-isoxazole) ring, all compounds were selective for the DAT relative to the 5-HTT and NET. No compound showed death when dosed at 100 mg/kg; however, most compounds did show signs typical of dopamine activity. The ED_{50} values for 2*β*-(1,2-isoxazoles) that caused locomotor stimulation ranged from 0.2 to 12.8 mg/kg. Most compounds showed slower on-set and longer duration of action relative to cocaine. Surprisingly, 3*β*-(4-methylphenyl)-2*β*-[3-(4′-chlorophenyl)isoxazol-5-yl]tropane (**3p**) and 3*β*-(4-methylphenyl)-2*β*-[3-(4′-methylphenyl)isoxazol-5-yl]tropane (**3r**) did not produce significant increases in locomotor activity. In the cocaine discrimination test, all analogues showed full or at least 50% generalization with the exception of **3p**, which did not show generalization. Importantly, both the locomotor activity and the cocaine discrimination ED_{50}values were correlated with the DAT binding but not 5-HTT and NET binding. This provides further support for the dopamine hypothesis of cocaine abuse. High DAT affinity and selectivity, increased locomotor activity with slow onset and long duration of action, and generalization to cocaine shown by the 3*β*-(substituted phenyl)-2*β*-(3-substituted isoxazol-5-yl)tropanes are properties thought necessary for a pharmacotherapy for treating cocaine abuse.

Introduction

We previously summarized the information that strongly suggested the dopamine transporter (DAT) as the cocaine receptor responsible in abuse, presented what was known about the site including structure activity relationship (SAR) information, speculated on a preliminary pharmacophore model, and provided perspectives for future research.[1] Many new studies by numerous investigators have been reported since this initial review, and the new results have been summarized in subsequent reviews.[2] After the synthesis of a few cocaine analogues, emphasis of our SAR efforts was directed toward 3-phenyltropane 2*β*-carboxylic acid methyl ester class of DAT uptake exhibitors.[2] These studies led to the discovery that 3*β*-(4-substituted phenyl)-2*β*-heterocyclic tropanes could function as bioisosteric replacements for the corresponding 3*β*-(4′-substituted phenyl)tropane-2*β*-carboxylic acid methyl esters.[3,4]



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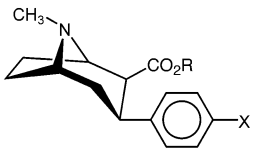
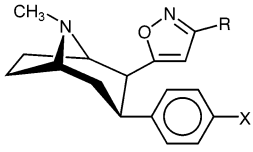


2a, X = H, R = CH_3
b, X = Cl, R = CH_3
c, X = CH_3, R = CH_3
d, X = CH_3, R = $(CH_3)_2CH$
e, X = Cl, R = C_6H_5



3a, X = Cl, R = CH_3
b, X = CH_3, R = CH_3
c, X = Cl, R = C_6H_5
d, X = CH_3, R = C_6H_5
e, X = Cl, R = H
f, X = CH_3, R =H
g, X = Cl, R = C_2H_5
h, X = CH_3, R = C_2H_5
i, X = Cl, R = $(CH_3)_2CH$
j, X = CH_3, R = $(CH_3)_2CH$
k, X = Cl, R = $(CH_3)_3C$
l, X = CH_3, R = $(CH_3)_3C$
m, X = Cl, R = $4FC_6H_4$
n, X = CH_3, R = $4FC_6H_4$
o, X = Cl, R = $4ClC_6H_4$
p, X = CH_3, R = $4ClC_6H_4$
q, X = Cl, R = $4CH_3C_6H_4$
r, X = CH_3, R = $4CH_3C_6H_4$
s, X = Cl, R = $4CH_3OC_6H_4$
t, X = CH_3, R = $4CH_3OC_6H_4$

One of the more interesting classes of 2*β*-heterocyclic analogues reported in these earlier studies was the 3*β*-

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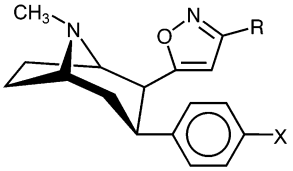
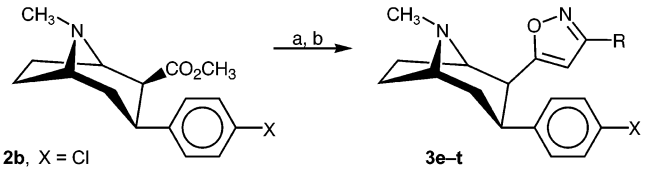
Table 1. 3β-(Substituted phenyl)tropane 2β-(3-Substituted isoxazol-5-yl)tropane Analogues, Yields, and Analytical Data



compd	X	R	yield[a,b] %	$[\alpha]^{25}_D$ (g/100 mL, CH_3OH)	mp (°C)	salt[c]	recrystallization[d] solvent	molecular formula	anal.
3e	Cl	H	23	−140.4 (0.45)	168 (dec)	*e*	$(CH_3)_2CHOH$	$C_{37}H_{37}ClN_2O_9$	C, H, N
3f	CH_3	H	28	−117.6 (0.42)	133 (dec)	*e*	$(CH_3)_2CHOH$	$C_{38}H_{40}N_2O_9 \cdot 0.5H_2O$	C, H, N
3g	Cl	C_2H_5	55	−99.14 (0.35)	232 (dec)	HCl	$CH_3OH/(C_2H_5)_2O$	$C_{19}H_{24}Cl_2N_2O \cdot 0.25H_2O$	C, H, N
3h	CH_3	C_2H_5	59	−99.47 (0.38)	235 (dec)	HCl	$CH_3OH/(C_2H_5)_2O$	$C_{20}H_{27}ClN_2O$	C, H, N
3i	Cl	$(CH_3)_2CH$	46	−92.86 (0.35)	230 (dec)	HCl	$CH_3OH/(C_2H_5)_2O$	$C_{20}H_{26}Cl_2N_2O$	C, H, N
3j	CH_3	$(CH_3)_2CH$	77	−90.9 (0.45)	232 (dec)	HCl	$CH_3OH/(C_2H_5)_2O$	$C_{21}H_{29}ClN_2O \cdot 0.25H_2O$	C, H, N
3k	Cl	$(CH_3)_3C$	42	−84.33 (0.30)	242 (dec)	HCl	$CH_3OH/(C_2H_5)_2O$	$C_{21}H_{28}Cl_2N_2O$	C, H, N
3l	CH_3	$(CH_3)_3C$	79	−87.4 (0.49)	239 (dec)	HCl	$CH_3OH/(C_2H_5)_2O$	$C_{22}H_{31}ClN_2O$	C, H, N
3m	Cl	$4FC_6H_4$	48	−109.0 (0.39)	244 (dec)	HCl	$CH_3OH/(C_2H_5)_2O$	$C_{23}H_{23}Cl_2FN_2O$	C, H, N
3n	CH_3	$4FC_6H_4$	35	−95.1 (0.69)	238 (dec)	HCl	$CH_3OH/(C_2H_5)_2O$	$C_{24}H_{26}ClFN_2O$	C, H, N
3o	Cl	$4ClC_6H_4$	41	−100.2 (0.51)	250 (dec)	HCl	$CH_3OH/(C_2H_5)_2O$	$C_{23}H_{23}Cl_3N_2O$	C, H, N
3p	CH_3	$4ClC_6H_4$	56	−100.9 (0.53)	237 (dec)	HCl	$CH_3OH/(C_2H_5)_2O$	$C_{24}H_{26}Cl_2N_2O$	C, H, N
3q	Cl	$4CH_3C_6H_4$	46	−102.9 (0.34)	260 (dec)	HCl	$CH_3OH/(C_2H_5)_2O$	$C_{24}H_{26}Cl_2N_2O \cdot 0.25H_2O$	C, H, N
3r	CH_3	$4CH_3C_6H_4$	51	−100.4 (0.70)	233 (dec)	HCl	$CH_3OH/(C_2H_5)_2O$	$C_{25}H_{29}ClN_2O$	C, H, N
3s	Cl	$4CH_3OC_6H_4$	70	−106.3 (1.2)	239 (dec)	HCl	$CH_3OH/(C_2H_5)_2O$	$C_{24}H_{26}Cl_2N_2O \cdot 0.75H_2O$	C, H, N
3t	CH_3	$4CH_3OC_6H_4$	56	−104.0 (0.48)	248 (dec)	HCl	$CH_3OH/(C_2H_5)_2O$	$C_{25}H_{29}ClN_2O_2$	C, H, N

[a] A typical experimental is given for each method. Details for the synthesis of each compound are given in Supporting Information. [b] This is the overall yield of the product. [c] The salt used to characterize the compound. [d] Solvent system used for recrystallization of the salt. [e] Di-*p*-toluoyl-L-tartrate.

Scheme 1[a]



2b, X = Cl
c, X = CH_3

3e–t

[a] Reagents: (a) $CH_3C(=NOH)R$, nC_4H_9Li, THF, 0 °C; (b) H_2SO_4, THF.

(4-substituted phenyl)-2β-(3-phenyl and 3-methylisoxazol-5-yl)tropanes (**3a**−**d**). In this paper, we describe the synthesis and monoamine binding properties of a number of new 2β-(3-substituted isoxazol-5-yl)tropanes analogues (**3e**−**t**) and present their behavioral pharmacology. Several of these compounds possess properties thought to be needed for a pharmacotherapy for treating cocaine abuse.

Chemistry

The synthesis of the 3β-(substituted phenyl)-2β-(3-substituted isoxazol-5-yl) tropanes (**3e**−**t**) is outlined in Scheme 1. A solution of the appropriate 3β-(4-chloro or 4-methylphenyl)tropane-2β-carboxylic acid methyl esters (**2b** or **2c**, respectively) was added to the dilithium salt of the appropriate ketone or aldehyde oxine in tetrohydrofuran (THF) at 0 °C under nitrogen, and the reaction mixture was allowed to warm to 25 °C. After a few hours, the reaction mixture was added to a THF solution containing sulfuric acid and refluxed for 1 h to give the desired products. The yield obtained along with the analytical data is given in Table 1.

Biology

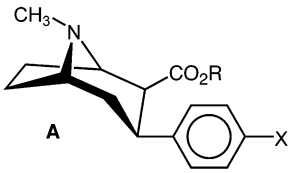
The binding affinities of the target compounds **3e**−**t** along with reference compounds **2d**, **2c**, cocaine, and WIN 35,065-2 at the DAT, serotonin transporter (5-HTT), and norepinephrine transporter (NET) were determined via competition binding assays using the previously reported procedures.[5] The results are listed in Table 2. The final concentration of radioligands in the assays was 0.5 nM [^{3}H]WIN35,428 for the DAT, 0.2 nM [^{3}H]paroxetine for the 5-HTT, and 0.5 nM [^{3}H]-nisoxetine for the NET.

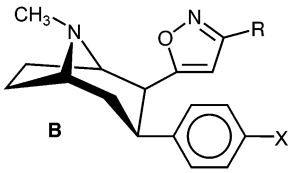
Behaviorally relevant doses for **3a**−**k** and **3m**−**t** were determined by observation of gross effects in mice given 1, 10, and 100 mg/kg (Table 3). Stimulant and/or depressant effects on locomotor activity were determined in mice (Table 4). Generalization with the cocaine cue was determined by lever choice in a drug discrimination task in rats. Table 5 shows the percent of rats choosing the cocaine lever at various doses of compounds along with ED_{50} values.

Results and Discussion

The results of the monoamine transporter binding studies are summarized in Table 2 along with results for cocaine, **2a** (WIN 35,065-2), and other previously reported compounds for comparison. We previously reported that modification of the 2β-carbomethoxy substituent of WIN 35,065-2 could lead to 3-phenyltropane analogues possessing selectivity for the DAT relative to the 5-HTT and NET. For example, the isopropyl and phenyl esters, **2d** and **2e**, both have high affinity and selectivity for the DAT (Table 2).[6,7] We also reported the synthesis of monoamine binding properties of a series of 3-phenyltropanes where the metabolically labile 3β-ester group was replaced by stable bioisosteric heterocyclic groups such as 1,2-isoxazoles (**3a**−**d**), 1,2,4-oxadiazoles (**4a**, **4b**, and **5**), 1,3,5-oxadiazoles (**6a**, **6b**, and **7**), 1,3,5-thiadiazoles (**8a**,**b**), 1,3-oxazoles (**9a**, **9b**), 1,3-thiazoles (**10**), and benzothiazoles (**11**).[3,4] A computational analysis of the electrostatic (molecular electrostatic potential), hydrophobic (calculated log *P*), and steric (substituted volume) properties of all 2β-heterocyclic moieties of the 3β-(4′-chlorophenyl) tropane ana-

Table 2. Monoamine Transporter Binding Properties of 3β-Phenyltropane 2β-Isoxazole Analogues



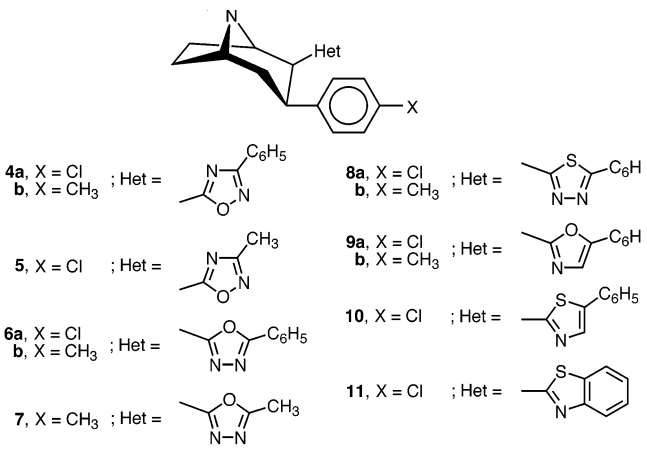


				IC_{50}, nM (K_i, nM)		
compd	X	structure	R	DAT [^{3}H]WIN 35,428	NET [^{3}H]nisoxetine	5-HTT [^{3}H]paroxetine
cocaine	–		–	89.1	3300 (1990)	1050 (45)
WIN 35,065-2	H	A	CH_3	23	920 (550)	2000 (182)
2d[a]	CH_3	A	$(CH_3)_2CH$	6.45 ± 0.85	6090 ± 488 (554 ± 44.3)	1930 ± 38 (1160 ± 22.9)
2e[a]	Cl	A	C_6H_5	1.98 ± 0.05	2340 ± 176 (213 ± 16)	2960 ± 223 (1780 ± 134)
3a[b]	Cl	B	CH_3	0.59 ± 0.04	181 ± 12 (109 ± 7.2)	572 ± 58 (52 ± 5.3)
3b[b]	CH_3	B	CH_3	0.93 ± 0.09	254 ± 31 (153 ± 19)	3820 ± 346 (348 ± 32)
3c[b]	Cl	B	C_6H_5	1.28 ± 0.18	2420 ± 135 (220 ± 2.6)	504 ± 29 (305 ± 18)
3d[b]	CH_3	B	C_6H_5	1.58 ± 0.02	398 ± 18 (239 ± 11)	5110 ± 187 (465 ± 42)
3e	Cl	B	H	0.77 ± 0.12	87 ± 7.9 (52 ± 4.7)	91 ± 13 (8.2 ± 1.2)
3f	CH_3	B	H	1.37 ± 0.03	43.4 ± 8.5 (26 ± 5.1)	414 ± 11 (38 ± 1.0)
3g	Cl	B	C_2H_5	0.50 ± 0.03	120 ± 10 (72 ± 6.3)	3090 ± 153 (281 ± 14)
3h	CH_3	B	C_2H_5	1.62 ± 1.0	299 ± 22 (180 ± 13)	6400 ± 253 (582 ± 53)
3i	Cl	B	$(CH_3)_2CH$	1.19 ± 0.12	954 ± 97 (575 ± 58)	2320 ± 153 (211 ± 14)
3j	CH_3	B	$(CH_3)_2CH$	4.5 ± 0.3	2520 ± 263 (1550 ± 24)	42 900 ± 3164 (3900 ± 355)
3k	Cl	B	$(CH_3)_3C$	7.31 ± 0.61	6320 ± 703 (3807 ± 424)	36 800 ± 3620 (3350 ± 329)
3l	CH_3	B	$(CH_3)_3C$	26 ± 1.4	3330 ± 144 (1825 ± 87)	295 000 ± 63 000 (26 000 ± 5700)
3m	Cl	B	$4FC_6H_4$	1.86 ± 0.09	918 ± 108 (553 ± 65)	7260 ± 210 (660 ± 19)
3n	CH_3	B	$4FC_6H_4$	6.45 ± 1.6	917 ± 30 (552 ± 18)	>100 000
3o	Cl	B	$4ClC_6H_4$	6.42 ± 0.46	5290 ± 448 (3790 ± 271)	>76 000
3p	CH_3	B	$4ClC_6H_4$	8.74 ± 1.7	6980 ± 1430 (3990 ± 716)	>100 000
3q	Cl	B	$4CH_3C_6H_4$	4.09 ± 0.44	1710 ± 39 (1030 ± 24)	5741 ± 421 (522 ± 48)
3r	CH_3	B	$4CH_3C_6H_4$	13 ± 2.1	>100 000	>100 000
3s	Cl	B	$4CH_3OC_6H_4$	1.57 ± 0.10	762 ± 38 (454 ± 23)	5880 ± 179 (535 ± 16)
3t	CH_3	B	$4CH_3OC_6H_4$	3.93 ± 0.49	756 ± 147 (455 ± 89)	4030 ± 31.3 (366 ± 2.9)

[a] Data taken from ref 7. [b] Data taken from ref 4.

logues with their DAT binding affinities strongly suggested a predominately electrostatic interaction for these 2β-heterocyclic analogues.

In the present study, we expanded the 3β-(1,2-isoxazoles) to include analogues **3e**−**t**. Compounds **3e** and **3f** have no substituent on the 1,2-isoxazole ring, compounds **3g**−**l** possess alkyl substituents, and **3m**−**t** have 4′-substituted phenyl ring substituents on the 2β-(1,2-isoxazole) ring. All the compounds are potent displacers (IC_{50} = 0.5 to 26 nM) for the DAT. With the exception of compounds **3e** and **3f**, which have no substituent on the 2β-(1,2-isoxazole) ring, all the compounds were selective for the DAT relative to the 5-HTT and NET. Compound **3r**, which has a 4-methylphenyl substituent on both the tropane and 1,2-isoxazole rings and has an IC_{50} value of 13 nM for the DAT and



>100 000 nM for both the 5-HTT and NET, is the most DAT selective analogue. The next most DAT selective analogues were **3k**, **3p**, and **3q**. Compounds **3a**, **3b**, **3f**, and **3g**, which have either no substituent or a small alkyl substituent on the 2β-(1,2-isoxazole) ring, show subnanomolar potency at the DAT (IC_{50} = 0.5 to 0.93 nM). However, **3b**, **3c**, **3d**, **3m**, and **3s**, which have phenyl or substituted phenyl ring substituents on the 2β-(1,2-isoxazole) ring, have IC_{50} values of less than 2 nM. In each case where both the 3β-chlorophenyl and 3β-methylphenyl analogues were evaluated, the 3β-chlorophenyl analogues showed higher affinity for the DAT than the corresponding 3β-methylphenyl analogue.

The gross observation and locomotor activity results in mice and the drug discrimination results in cocaine-trained rats for compounds **3a**−**t** and cocaine are shown in Tables 3, 4, and 5, respectively, except that no behavioral data were generated for **3l**. In addition to providing dosing guidance for the locomotor activity studies, gross observation allowed an overall determination of the effects on mice at three doses, with two mice per dose. It is important that no deaths were observed with any of the compounds even at 100 mg/kg, where cocaine is sometimes lethal. At 1 mg/kg, **3a**, **3c**, **3d**, **3e**, **3g**, **3i**, **3m**, **3n**, **3q**, **3s**, and **3t** produced stereotypy, hyperactivity, or stimulation. Compound **3p** caused hypoactivity, and **3m** caused circling. Cocaine, **3b**, **3f**, **3h**, **3j**, **3k**, **3o**, and **3r** had no effect at 1 mg/kg. At 10 mg/kg, cocaine and all compounds produced stereotypy, hyperactivity, or stimulation except for **3o**, which caused slight and intermittent bouts of both hyper- and hypoactivity, **3p**, which caused hypoactivity,

Table 3. Compounds **3a**−**k** and **3m**−**t** in Mice (ip): Gross Signs over 4 h

compd	1 mg/kg	10 mg/kg	100 mg/kg
cocaine		HA	C,Sy,HA
3a	Sy,HA	Sy,HA(sl),ST	C,Sy,Ho,HL,ST
3b		Sy,HA	C,Sy,HA,HL,ST
3c	HA(sl)	Sy,HA,Cc	Sy,Ho,HA,Cc
3d	HA(sl)	Sy,HA	Sy,Ho,Cc
3e	Sy(sl)	Sy,HA,Cc	Sy,HA,Cc,ST
3f		Sy,HA,Cc,T	Sy,HA,Cc,HL,T,ST
3g	Sy,HA	Sy	C,Sy,A,HL
3h	EG	Sy,HA,Cc	Sy,Ho,HL
3i	HA(sl)	Sy,HA	Sy,Cc,T,A,HL,FBP
3j		HA	Sy,A,FBP
3k		Sy,HA	C,Sy,A,HL,T,FBP
3m	HA(sl),Cc	Sy,HA(sl),Cc	Sy,Cc
3n	Sn(sl)	Sy(sl),Sn	Sy(sl),Ho,Cc
3o		Ho(sl),HA(sl)	Sy,Ho,Cc
3p	Ho	Ho,Cc	Ho,Cc,MR
3q	Sn	HA(sl)	Sy,Ho,HA,Cc
3r			Ho,Cc,P,MR
3s	HA(sl)	Sy,HA	Sy,Cc
3t	Sn(sl)	Sy,Cc,Sn	Sy,Cc

A	ataxia
C	convulsions
Cc	circling
EG	excessive grooming
FBP	flattened body posture
HA	hyperactivity
HL	hind limb splay
Ho	hypoactivity
MR	muscle relaxation
P	ptosis
Sn	stimulation
ST	Straub tail
Sy	stereotypy
T	tremor
(sl)	slight or intermittent

and **3r**, which had no effect. In addition to stimulation, **3b**, **3e**, **3f**, **3h**, **3m**, and **3p** caused circling. Compound **3e** caused tremors, and **3a** caused Straub tail. At 100 mg/kg, cocaine and all compounds, except **3p** and **3r**, produced stereotypy. Cocaine, **3b**, **3c**, **3e**, **3f**, and **3q** caused hyperactivity. For most of the other compounds, 100 mg/kg produced no hypoactivity or change probably because of the intense stereotypy and circling. Most of the additional symptoms at 100 mg/kg were in compounds without the phenyl substitution on the isoxazole ring. Cocaine, **3a**, **3c**, **3g**, and **3k** caused convulsions. Tremor was seen with compounds **3f**, **3i**, and **3k**; Straub tail with **3a**, **3c**, **3e**, and **3f**; hind limb splay with **3a**, **3c**, **3f**, **3g**, **3h**, **3i**, and **3k**; flattened body posture with **3i**, **3j**, and **3k**; ataxia with **3g**, **3i**, **3j**, **3k**; ptosis with **3r**; and muscle relaxation with **3p** and **3r**.

Table 4 shows locomotor activity in mice in 1-h bins over 4 h. Cocaine produced its greatest stimulation in Hour 1, and by Hours 3 and 4 the effect was gone. In contrast, all 2β-(isoxazoles) that produced stimulation, except **3e** and **3f**, had their largest effect in Hours 2, 3, or 4. Compounds **3e** and **3f**, like cocaine, had their largest effect in Hour 1, but, unlike cocaine, increased activity through Hours 3 (**3e**) or 4 (**3f**). Surprisingly, two compounds, **3p** and **3r**, did not produce significant increases in locomotor activity at any dose tested. The ED_{50} value for stimulation by cocaine in Hour 1, its time of peak effect, was 18.8 mg/kg. The ED_{50} values for the 2β-(1,2-isoxazoles) that caused locomotor stimulation ranged from 0.2 to 12.8 mg/kg. Not only were the 2β-(1,2-isoxazoles) more potent than cocaine in their peak effect hour, but most produced a greater stimulation than cocaine during Hour 1. The correlation between the ED_{50} values in the peak hour of locomotor stimulation and potency at the DAT was $r = 0.56$ ($p < 0.02$); the correlations with NET and 5-HTT were lower and nonsignificant. Interestingly, the correlation between the Hour 1 ratio to cocaine and potency at the DAT was $r = -0.70$ ($p < 0.002$). Thus, in general, the 2β-(1,2-isoxazoles) are more potent than cocaine in causing locomotor stimulation and have a longer duration of action. These results are not unexpected given that other 3β-phenyltropanes have been reported that are more potent and have a longer duration of action as locomotor stimulants than cocaine.[8−10]

Table 5 shows effects in rats trained to discriminate cocaine. Thirteen of the 19 compounds showed full generalization (≥75% of rats choosing the cocaine lever) to the cocaine cue, with ED_{50} values ranging from 0.19 to 11.0 mg/kg; the ED_{50} value for cocaine was 2.64 mg/kg. Of the remaining six compounds, five (**3i**, **3m**, **3o**, **3r**, and **3s**) showed at least 50% generalization, but severe side effects precluded testing higher doses. Compound **3p** stood out in that it showed no generalization. For the compounds that generalized, the correlation between the ED_{50} value in drug discrimination and binding to the DAT was $r = 0.59$ ($p < 0.05$); correlations to NET and 5-HTT were lower and nonsignificant. Generalization to the cocaine cue in rats by 3β-phenyltropanes has been previously reported.[10,11]

In summary, several 3β-(substituted phenyl)-2β-(3-substituted isoxazol-5-yl)tropanes were synthesized and evaluated for their ability to inhibit radioligand binding at the DAT, 5-HTT, and NET. Compounds with no substituent (**3e** and **3f**), small to large alkyl substituents (**3a**, **3c**, **3g**−**l**), and phenyl and 4-substituted phenyl substituents (**3d**, **3m**−**t**) on the 2β-isoxazole ring showed high affinity for the DAT. Compounds **3c**, **3f**, and **3g** showed subnanomolar affinity for the DAT; however, several other analogues with phenyl and substituted phenyl substituents on the 2β-isoxazole ring (**3c**, **3d**, **3m**, and **3s**) have IC_{50} values of less than 2 nM. With the exception of **3e** and **3f**, all analogues were selective for the DAT relative to the 5-HTT and NET. In gross observation, none of the compounds produced death at 100 mg/kg; however, cocaine and **3a**, **3c**, **3g**, and **3k** produced convulsions at this dose. With the exception of **3p** and **3r**, all analogues produced stimulation of locomotor activity, with ED_{50} values from 0.2 to 12.8 mg/kg; by comparison, cocaine's ED_{50} was 18.8 mg/kg. With the exception of **3a** and **3f**, all analogues had a slower onset and longer duration of locomotor activity stimulation than cocaine. Most produced stimulation equal to or slightly greater than cocaine. Compound **3g**, with stimulation 2.1 times that of cocaine, and **3o**, with stimulation only 0.4 times that of cocaine, are exceptions. In the cocaine discrimination test, all analogues showed full or at least 50% generalization with the exception of **3p**, which showed none. Importantly, both the locomotor activity and the cocaine discrimination ED_{50} values were correlated with the DAT binding, but not 5-HTT and NET binding. This provides further support for the dopamine hypothesis of cocaine abuse.

Because several 2β-(1,2-isoxazole) analogues are DAT selective, increase locomotor activity with slow onset

Table 4. Percent Change from Vehicle in Locomotor Activity in Mice (ip) for Compounds **3a**−**k** and **3m**−**t**

Compd	Dose mg/kg	Hour 1	Hour 2	Hour 3	Hour 4	ED_{50} in Peak Hour mg/kg	First Hour: Ratio to Cocaine
Cocaine	5.6	+5	-26	-53	-1	18.8	
	17	+179 *	-7	-53	-26		
	56	+452 *	+201 *	-47	-24		
3a	0.1	+48	+92	+38	-23	0.2	1.2
	0.3	+354 *	+526 *	+735 *	+392 *		
	1	+543 *	+434 *	+926 *	+981 *		
3b	0.3	+140 *	+156 *	+75	+48	1.1	1.1
	1	+341 *	+413 *	+392 *	+78		
	3	+478 *	+552 *	+986 *	+221		
3c	1	+11	+27	+45	+76	3.4	1.1
	3	+331 *	+456 *	+377 *	+378		
	10	+513 *	+402 *	+485 *	+924 *		
3d	1	+48	+139	+89	+154	2.5	1.2
	3	+244 *	+369	+283	+329		
	10	+526 *	+663 *	+363	+141		
3e	0.3	+57	+86	+70	-20	1.2	1.1
	1	+186 *	+284 *	+345 *	+182 *		
	3	+486 *	+137	-40	-71		
3f	0.3	-13	-12	+136	-28	1.0	1.5
	1	+319 *	+214 *	+48	-32		
	3	+698 *	+666 *	+571 *	+125		
3g	0.1	+82	+224	+184	+103	0.4	2.1
	0.3	+284 *	+505 *	+224	+208		
	1	+954 *	+1900 *	+1708 *	+778 *		
3h	0.3	+34	+99	+173	-72	0.9	1.4
	1	+449 *	+489 *	+222	+48		
	3	+613 *	+893 *	+725 *	+81		
3i	0.3	+34	+41	-21	-39	0.8	1.9
	1	+370 *	+632 *	+188	-2		
	3	+876 *	+995 *	+680 *	+1745 *		
3j	1	-1	+29	-20	+24	4.7	0.9
	3	+329 *	+241 *	+207	+70		
	10	+637 *	+532 *	+765 *	+576 *		
3k	1	+41	+103	+135	+33	4.0	0.9
	3	+112 *	+204 *	+206	-29		
	10	+388 *	+769 *	+1272 *	+229 *		
3m	1	-26	+38	-77	+37	2.8	1.6
	3	+271 *	+463 *	+479 *	+392		
	10	+729 *	+832 *	+514 *	+767		
3n	3	+105	+80	+148	+140	6.3	0.4
	10	+117	+268	+84	+148		
	30	+192	+383 *	+387 *	+179		
3o	1	+15	+19	+74	+39	3.6	0.4
	3	+59	+237 *	+298	+389		
	10	+166 *	+612 *	+838 *	+1372 *		
3p	0.3	-35	-20	-58	+45	No Stimulation	
	1	+5	+24	+33	+27		
	3	+30	+39	+7	-1		
	10	-1	-3	-45	-20		
	30	-26	+38	+79	+52		
3q	1	+8	+44	-49	+170	3.9	0.7
	3	+23	+153	+265	+119		
	10	+308 *	+1034 *	+1024 *	+493		
3r	0.3	-17	+60	+102	-48	No Stimulation	
	1	-9	-6	+76	-30		
	3	+1	+10	+73	+29		
	10	-30	-34	-38	-7		
	30	-19	-26	-37	-43		
3s	1	0	+51	+101	-21	3.7	1.2
	3	+197 *	+481 *	+662 *	+335		
	10	+521 *	+1337 *	+2830 *	+2120 *		
3t	3	+73	+32	+27	+30	12.8	0.6
	10	+187 *	+115 *	+65	+34		
	30	+290 *	+235 *	+430 *	+483 *		

* = Different from vehicle by Newman-Keuls following One-Way Analysis of Variance, $p < 0.05$.

and long duration of action, and generalize with cocaine, they have properties thought necessary for the treatment of cocaine abuse. It is extremely interesting that the highly DAT selective **3p** does not increase locomotor activity or generalize with cocaine. This could be due to lack of brain penetration. However, it could also be due to interaction at some other site. Lack of a cocaine-like effect of compounds that bind with a high affinity to the DAT has been previously reported.[12] Additional studies will be required to determine the reason for the unusual properties of **3p**.

Experimental Section

Proton and carbon-13 nuclear magnetic resonance (^{1}H NMR) and ^{13}C NMR spectra were recorded on either a 250 MHz (Bruker AM-250) or a 300 MHz (Bruker AVANCE 300) spectometer. Chemical shift data for the proton resonances were reported in parts per million (δ) relative to internal $(CH_3)_4Si$ (δ 0.0). Optical rotations were measured on an AutoPol III polarimeter, purchased from Rudolf Research. Elemental analyses were performed by Atlantic Microlab, Norcross, GA. Analytical thin-layer chromatography (TLC) was carried out on plates precoated with silica gel GHLF (250 μm thickness). TLC visualization was accomplished with a UV lamp or in an iodine chamber. All moisture-sensitive reactions were performed under a positive pressure of nitrogen maintained by a direct line from a nitrogen source.

THF was distilled just prior to its use (sodium benzophenone ketyl) or was purchased.

General Procedures for the Synthesis of 3β-(4-Substituted phenyl)-2β-(3-substituted isoxazol-3-yl)tropanes (3e−t). A representative experimental procedure is given below. The percent yields, salts prepared, recrystallization solvents, and analytical data for each compound are given in Table 1.

3β-(4-Chlorophenyl)-2β-(3-(4′-methylphenyl)isoxazol-5-yl)tropane Hydrochloride (3q, RTI-336). A solution of *n*-butyllithium in hexane (1.6 M, 3.95 mL, 6.33 mmol) was added to a stirred solution of 4′-methylacetophenone oxime (0.473 g, 3.17 mmol) in dry THF (8 mL) at 0 °C (N_2 atm). Upon addition of the lithium base, the reaction mixture was allowed to warm to room temperature and stirred for 2 h. The reaction mixture was recooled to 0 °C, followed by the addition of **2b** (0.750 g, 2.44 mmol) dissolved in THF (8 mL). The reaction mixture was then warmed to room temperature over 18 h.

The mixture was poured into a stirred solution of concentrated H_2SO_4 (3.2 g), THF (15 mL), and water (4 mL), which was refluxed for 1 h. Upon completion of refluxing, the cooled reaction mixture was made basic using saturated aqueous K_2CO_3 and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo to give an orange residue. Purification by flash chromatography on silica gel using 20% $(C_2H_5)_2O$:$C_2H_5)_3N$ (9:1)/(80%) CH_2Cl_2 gave a white solid. Recrystallization from ether/petroleum ether produced 0.480 g (46%) of product as white needles: ^{1}H NMR ($CDCl_3$) δ 7.66 (d, J = 8.0 Hz, 2H),

Table 5. Drug Discrimination Effects of Compounds **3a**−**k** and **3m**−**t** in Rats (ip)

	percent of rats choosing the cocaine lever at dose (mg/kg)												
	0.1	0.17	0.3	0.56	1	1.7	3	5.6	10	17	30	ED_{50}, mg/kg	termination symptoms
compd													
cocaine					21	32	59	82	97			2.64	
3a	0	25	88									0.21	
3b	13	0	83									0.28	
3c							29	63	75			4.73	
3d							0	75	86			4.88	
3e	13	29	63	25	100							0.41	
3f	0	43	83		100							0.19	
3g			0	75	71							0.48	A
3h			0	14	75	100						0.82	
3i					13	25	63						A,B
3j							25	67	86			4.63	
3k					0		50	88	83			3.04	
3m							13	43	63				A,D
3n						0		25	25	88		7.27	
3o					0		13	0	50				A,C
3p					0		13		0		0		F
3q					14		14	50	88			5.95	
3r							0		0	33	67		E,F
3s					0		0	43	50				A
3t								0		43	83	11.00	

reasons for terminating the dose−response curve below 80% choice:
A = severe stereotypy during or after the session interfering with eating
B = irritation at injection site
C = severe hyperactivity during session
D = self-mutilation
E = twenty-five percent or more of rats made fewer than 10 responses on a single lever
F = pronounced muscle relaxation

7.22 (d, J = 8.0 Hz, 2H), 7.12 (d, J = 8.5 Hz, 2H), 6.94 (d, J = 8.5 Hz, 2H), 6.76 (s, 1H), 3.15−3.44 (m, 4H), 2.33 (s, 3H), 2.01−2.31 (m, 6H), 1.59−1.91 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 1.73.2, 161.6, 140.1, 139.5, 132.1, 129.3, 128.7, 128.2, 126.8, 126.6, 101.4, 65.4, 61.6, 46.1, 41.9, 35.5, 34.9, 26.4, 25.0, 21.3.

The freebase was converted to the hydrochloride salt and recrystallized from MeOH/ether: mp 214 °C (dec); $[\alpha]^{25}_D$ −102.9° (*c* 0.34, CH_3OH); ^{1}H NMR (CD_3OD) δ 7.57 (d, 2H), 7.18−7.45 (cp, 6H), 6.20 (s,1H), 4.30 (d, 1H), 4.19 (m, 1H), 4.01 (m, 1H), 3.72−3.90 (cp, 1H), 2.92 (s, 3H),2.10−2.90 (cp, 9H). Anal. ($C_{24}H_{26}Cl_2N_2O\cdot 0.25H_2O$): C, H, N.

Gross Observation. Subjects. Male CD-1 mice, 19−28 g, from Charles River Laboratories, Raleigh, NC, were habituated to the Animal Research Facility for at least 5 days.

Apparatus and Procedure. Observations were made in six clear Plexiglas chambers, 4 in. × 6 in. by 4 in. Compounds were homogenized in 0.5% methyl cellulose and injected at 1, 10, or 100 mg/kg ip in a volume of 10 mL/kg of body weight (N = 2 per dose in the same chamber). Control mice received vehicle. Subjects were observed continuously for the first 15 min and thereafter at 1/2, 1, 2, 4, and 24 h after injection. Gross signs such as hyperactivity, hypoactivity, stereotypy, blepharoptosis, seizure, and death were recorded in narrative form with indications of presence and intensity.

Locomotor Activity. Subjects. Male CD-1 mice, 19−28 g, from Charles River Laboratories, Raleigh, NC, were habituated to the Animal Research Facility for at least 5 days.

Apparatus and Procedure. Activity was measured in 24 plexiglass chambers, 16 in. × 8 in. × 8 in, each set in an array of four photocells in a Cage Rack system (San Diego Instruments, San Diego, CA). Initial doses were selected on the basis of gross observation results, with 30 mg/kg being the upper limit. Compounds were prepared as for gross observation. Mice (N = 5 or 6 per dose and vehicle, with a replication to increase the N for compounds of particular interest or to extend the dose−effect curve) were habituated to the activity chambers for 1/2 h, then removed individually, injected ip, and replaced. Photobeam interruptions were recorded in 10-min bins for 4 h.

Analyses. Data were grouped into 1-h time bins and subjected to one-way analysis of variance, with the Newman-Kuels test applied post hoc at each time point where a main effect of dose or a dose × time interaction was significant ($p < 0.05$). An ED_{50} was determined for the hour showing the greatest change from control by using a sigmoidal dose−response (variable slope) curve-fitting procedure (GraphPad Prism, GraphPad Software, Inc., San Diego, CA).

Drug Discrimination. Subjects. Adult male CD albino rats (Sprague Dawley derived) from Charles River Laboratories, Raleigh, NC, were maintained one or two per cage on a reverse light/dark cycle (lights on 1800 to 0600) in an animal housing room with controlled temperature (69 to 75 °F) and humidity (40 to 70%).

Apparatus and Procedure. Training and testing were done in 16 standard rat operant chambers inside sound-attenuating enclosures (Coulbourn Instruments, Allentown, PA). Each chamber was equipped with a house light, two response levers, a food trough between the levers, and a dispenser for 45-mg food pellets (BioServ, Frenchtown, NJ). Programming of contingencies and data acquisition were done by an L2T2 system (Coulbourn Instruments).

Initial Training. Subjects were deprived of food for 24 h and given at least two 1-h sessions on an autoshaping schedule and at least two 15-h nightly sessions on a progressive ratio schedule.

Cocaine Discrimination Training. When subjects readily emitted 10 lever presses for each food pellet (FR10), they were put on daily 10-min sessions on FR10 for 5 days per week, with access to standard lab chow postsession and on Saturday and Sunday in sufficient quantity to maintain good health and stable body weight. Fifteen minutes before each session, either cocaine 10 mg/kg or saline vehicle was injected ip in a volume of 1 mL/kg of body weight. For half of the subjects, after cocaine injection, every 10 presses on the left lever delivered a pellet and presses on the right lever had no programmed consequence; after saline injection, every 10 presses on the right lever delivered a pellet and presses on the left lever had no programmed consequence. For the other half of the subjects, this contingency was reversed. A correct lever choice for a session was defined as earning the first pellet with 12 or fewer presses (i.e., no more than two on the incorrect lever). Cocaine (C) or saline (S) was assigned such that each was given on 2

consecutive days (e.g., for the 5 days of 1 week, C−C−S−S−C, and for the next week, C−S−S−C−C). The criterion for stability on the discrimination was at least nine correct choices for the first FR10 in 10 consecutive sessions. To maintain stability, a subject had to have made the correct choice in the most recent drug-lever-correct and the most recent saline-lever-correct sessions.

Compound Testing for Generalization with the Cocaine Cue. Days were assigned to cocaine, saline, and test compound (T) in a double alternation sequence such that a T day occurred 3 times every 2 weeks, with sequential T days separated by at least 1 C and 1 S day. A compound was tested initially at doses that produced stimulation in mouse locomotor activity, and then a full dose−effect curve from 20% or less to 80% or greater generalization was generated. The highest dose was that which reduced lever pressing by >50%, or produced significant side effects, or 30 mg/kg, whichever was lower. Degree of generalization was defined as the percentage of subjects that chose the cocaine-appropriate lever. In most cases, all doses of a compound were tested in seven or eight subjects. Compounds were prepared as for the mouse experiments and injected ip in a volume of 1 mL/kg 15 min before the session.

Analyses. Lever choice on a test day was defined as the first lever on which 10 presses occurred. Total presses for the session also were recorded. Results for a compound were tabulated as the percent of subjects choosing the cocaine lever. Where cocaine-lever choice reached 75%, an ED_{50} was calculated with GraphPad Prism.

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Supporting Information Available: Detailed experimental procedures for the synthesis of **3e**−**t**. This material is available free of charge via the Internet at http://pub.acs.org.

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